

The tadpole of *Eupsophus queulensis* (Anura, Cycloramphidae)

Diana R. CÁRDENAS-ROJAS*, Alberto VELOSO** & Rafael O. DE SÁ***

* Instituto de Zoología,
Universidad Austral de Chile, Casilla 567, Valdivia, Chile <diandrobates@gmail.com>

** Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile,
Casilla 653, Santiago, Chile

*** Department of Biology, University of Richmond, Richmond, 23173 Virginia, USA

The genus *Eupsophus* is characterized by endotrophic larvae that inhabit water-filled cavities. The larvae feed from large yolk reserves during metamorphosis. The external morphology has been described for four of the ten known species and data on larval chondrocranial morphology are available only for one. We describe the external morphology, oral disc and chondrocranial anatomy of the larvae of *Eupsophus queulensis*. The characteristics of *E. queulensis* tadpoles are compared with those of other *Eupsophus* larvae.

INTRODUCTION

The genus *Eupsophus* consists of 10 recognized species, including the recently described *Eupsophus queulensis* (VELOSO et al., 2005). They are distributed throughout the temperate forests of southern Chile and Argentina. The larvae of only four species of *Eupsophus* have been described so far: *E. roseus* (FORMAS & PUGÍN, 1978), *E. emihopagum* (FORMAS, 1989a), *E. calcaratus* (FORMAS, 1989b; VLKA CANDIOTTI et al., 2005) and *E. vertebralis* (FORMAS, 1992). These larvae have been classified as endotrophic-micicolous (ALFUG & JOHNSTON, 1989). They are characterized by having few denticles, scarce pigmentation, and internal organs visible through the unpigmented ventral skin.

Herein, we describe and illustrate the external morphology of the tadpole of *Eupsophus queulensis* and its chondrocranial anatomy, comparing the characteristics of this species with those previously described for the genus.

MATERIALS AND METHOD

Alberto Veloso and Klaus Busse collected a clutch of eggs, along with a guarding adult female, from a water-filled cavity at the type locality, Reserva Nacional Los Queules (35°59'S,

72°41'W), Maule Region, Chile, on November 2003. The female was included among the type series in the description of the species (VELOSO et al., 2005). The eggs were brought and hatched in the laboratory. Four larvae developed and they were used in this description. Specimens were fixed in 95% ethanol and subsequently preserved in 10% commercial grade formalin. Tadpoles are in Gosner stage 34 (GOSNER, 1960).

Measurements, terminology and labial tooth row formula follow ALTIG & MCDIARMID (1999). Measurements were taken using an eye micrometer under a Wild M3C Leica stereomicroscope. The description of larval external morphology is based on the examination of four larvae (Cárdenas-Rojas Field Number DRCR 062). After examination of external morphology, two larvae were prepared for scanning electron analysis. The other two larvae were deposited at the herpetological collection of the Instituto de Zoología Universidad Austral de Chile (IZUA), Valdivia, Chile. Tadpole illustration is based on individual IZUA 3708. The other specimen was used for analysis and description of chondrocranial anatomy (IZUA 3709); the specimen was cleared and double-stained for bone and cartilage using a modified technique of DINGERKUS & UHLER (1977). Chondrocranial terminology follows HAAKS (1995, 2003) and LARSON & DE SÁ (1998). Illustrations of larval morphology and chondrocranial anatomy were made using a Wild M3C Leica stereomicroscope with a camera lucida attachment.

RESULTS

DESCRIPTION OF TADPOLE

Tadpoles of *Eupsophus queulensis* have an elliptical body (fig. 1). The snout is rounded in dorsal and lateral views, the dorsal contour of the body gradually curved from mid-body to snout. The nostrils are situated midway between the tip of snout and the anterior border of eyes. The eyes are large, they are laterally positioned and directed anterodorsally, they are separated by a distance $1.9 \times$ the eye diameter. The spiracular tube is absent, the spiracular opening is small, lateral and sinistral. The length of the tail is nearly twice the body length, the dorsal and ventral fins are well developed and almost parallel to the tail musculature, the dorsal fin originates on the posterior third of the body and the ventral fin originates posterior to the vent tube. The caudal musculature is moderately robust with poorly defined myotomes; the tail tip is rounded. The vent tube and opening are medial. The color of preserved specimens is yellowish white whereas the abdomen and fins are transparent and the internal organs are visible. The dorsal surface area is light brownish with scarce and minute melanophores.

The oral disc is positioned ventrally, it is not emarginated and it possesses a single row of marginal papillae with a wide rostral gap (about 1/3 of the anterior labium), submarginal papillae are absent (fig. 2). The labial tooth row formula is 2(2)/2, the posterior labial tooth rows are about equal in length. The upper and lower jaw sheaths are pigmented for about one-third of their width and have serrated edges.



Fig 1 - Lateral view of *Eupsophus queulensis*, IZUA 3708, Gosner stage 34 tadpole. Bar 5.0 mm



Fig 2 Oral disc of *Eupsophus queulensis*, IZUA 3708, Gosner stage 34 tadpole Bar 0.5 mm

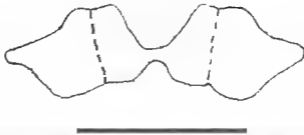


Fig. 3 - Suprarostral cartilages of *Eupsophus queulensis*, IZUA 3709, Gosner stage 34 tadpole. Bar 0.5 mm

Measurements in millimeters of tadpole IZUA 3708, following AL TIG & MCDIARMID'S (1999) methodology, are as follows: body length 6.3; body height 3.7, body width 4.2, tail length 12.0; maximum tail height 4.5; tail muscle height 2.2; tail muscle width 1.9; interorbital distance 2.9; internarial distance 1.6, oral disc length 1.9.

CHONDROCRANIUM

The paired suprarostral cartilages support the upper horny beak. Each suprarostral consists of a central body and a lateral wing. The bodies of the suprarostals are vertically rectangular and they are largely fused to the wings (fig. 3). The bodies of the suprarostals are connected ventromedially via a narrow cartilage. The lateral wings of the suprarostals are triangular-shaped; the width of each suprarostral's wing is about twice that of the body, the wings have a short but clearly visible *processus (p) posterior dorsalis*. The suprarostral bodies and the medial margins of the suprarostals' wings articulate with the tip of the trabecular horns.

The *cornua trabeculae* are short, less than 20% of the total chondrocranial length. They project anteriorly from the *planum trabeculare anticum*, diverging from each other and forming a wide and shallow "U". The *cornua trabeculae* are almost uniform in width throughout their length and they curve strongly ventrally to articulate with the suprarostals (fig. 4A, C). The *p. lateralis trabeculae* is poorly developed and almost indistinguishable; it connects laterally the *cornua trabeculae* with the *p. quadratoethmoidalis* via the *ligamentum quadratoethmoidale*. The posterior confluence of the *cornua trabeculae* is continuous with the *planum trabeculare anticum* and the developing *planum ethmoidale*, the later begins to form the anterior wall of the braincase at this stage. The *tectum nasi* has not yet begun to develop.

At stage 34, the cranial floor is not yet fully chondrified, a thin cartilage closes the *fenestra basiscranialis*. Two sets of openings are found on the cranial floor. The anterior pair, the *foramina craniopalatina*, is small and almost not visible, whereas the posterior pair, the *foramina carotica primaria*, is well defined (fig. 4B).

The *cartilago orbitalis* forms the lateral walls of the braincase. However, these cartilages do not connect to the otic capsule yet, consequently, the dorsal and anterior margins of the

foramen (f) prooticum are open (fig. 4C). The *f. opticum* and the *f. oculomotorium* perforate the *cartilago orbitalis*, the later being the smaller of the two and found posterior and ventrally to the former.

The *fenestra frontoparietalis* is not yet complete. Laterally, we found very thin *taeniae tecti marginales*, and posteriorly the *tectum sinoticum* is found as a narrow cartilage connecting the otic capsules (fig. 4A).

The otic capsules are about 33% of the total chondrocranial length and 38% of the total chondrocranial width. The ventrolateral surface of the otic capsules bears a large *fenestra ovalis*. A larval *crista parotica* and *p. oticum* are absent. The *arcus occipitalis* extends ventrally from the posteromedial margin of the otic capsule, fusing with the *planum basale* and giving rise to the occipital condyles. The *f. perilymphaticum inferior* is found lateral to the *f. jugulare* on the ventromedial margin of the otic capsule (fig. 4B).

The *cartilago palatoquadrati* has a constant width throughout its length. It has two attachments to the braincase, anteriorly the *commissura quadrato cranialis anterior* and posteriorly the *p. ascendens*. The *commissura quadrato cranialis anterior* extends between the *cartilago palatoquadrati*, at a level just posterior to the *pars articularis quadrati*, and the floor of the neurocranium (fig. 4A-B). The anterior margin of the *commissura quadrato cranialis anterior* bears a triangular *p. quadratoethmoidalis*, which serves as the point of attachment for the *ligamentum quadratoethmoidale*. A very short, almost indistinct, and blunt *p. antorbitalis* is present. The posterior curvature of the *cartilago palatoquadrati* extends slightly beyond the level of attachment of the *p. ascendens* to the braincase. The lateral and posterior margins of the palatoquadrate curve slightly dorsally, giving the palatoquadrate a concave appearance in dorsal view. The *p. ascendens* is a narrow and rod-like cartilage connecting the posteromedial margin of the *cartilago palatoquadrati* to the *cartilago orbitalis*. The *p. ascendens* attaches just posterior to the *oculomotor foramen*, i.e., SOKOL's (1981) intermediate condition. The *p. ascendens* is almost perpendicular (85-90° angle) to the main axis of the chondrocranium (fig. 4A-C).

Anteriorly, the *cartilago palatoquadrati* has two processes, the *p. muscularis quadrati* and the *pars articularis quadrati*. The *p. muscularis* is broad, flat, and extends dorsally from the lateral margin of the *cartilago palatoquadrati* posterior to the *pars articularis quadrati*. The *p. muscularis* is visible in lateral view. The dorsal edge of the *p. muscularis* inclines medially; a *commissura quadratoorbitalis* is absent. Immediately below the *p. muscularis* and ventrally on the *cartilago palatoquadrati* there is a notch, the *facies articularis hyalis*, which serves as the point of articulation of the ceratohyal with the *cartilago palatoquadrati*. On the articular surface is the hyoquadrate process which in lateral view is a small and sub-triangular process. The *pars articularis quadrati* is slightly angled medially and articulates broadly with the *cartilago Meckeli*.

Meckel's cartilages, together with the infraorbital cartilages, form the mandible during larval stages. Meckel's cartilage is stout and has a sigmoid-shape; its anteromedial margin is concave whereas its anterolateral margin is convex. Laterally, the *cartilago Meckeli* articulates with the *pars articularis quadrati* via the *p. retroarticularis*. This process curves ventrally beneath the *pars articularis quadrati*. Anteromedially, the *cartilago Meckeli* has two processes, the *p. dorsomedialis* and the *p. ventromedialis*. The posterodorsal portion of the infraorbital cartilages articulates with Meckel's cartilages between these two processes. The infraorbital

cartilages provide support for the lower keratinized beak. Each infraorbital is rectangular in shape and outwardly rounded over their entire anterior margin.

HYOBANCHIAL APPARATUS

The *ceratohyal* are medially wide and flat cartilages; laterally, they are twisted dorsally to articulate with the *cartilago palatoquadrati* at the *facies articularis hyalis*. Each *ceratohyal* bears two processes on the anterior margin, a triangular *p. anterior hyalis* and a rounded and small *p. anterolateralis hyalis*. The *ceratohyalia* also have a well-developed *p. posterior hyalis*. Medially, the *ceratohyalia* are connected by a *pars reunens*, which is continuous with the *copula posterior*. Posteroventrally, the *copula posterior* has a small *p. urobranchialis*. The *copula posterior* is continuous with the hypobranchial plates. The hypobranchial plates do not contact each other medially; they are continuous with the ceratobranchials (Cb). Posteriorly, the hypobranchial plates diverge and their posterior edges form an inverted V (fig. 4D).

The branchial baskets consist of four ceratobranchials (Cb I-Cb IV). The *p. branchialis* is absent. The ceratobranchials are distally continuous via the *commisurae terminales*, except between Cb I and Cb II. Proximally, ceratobranchial I is attached to the hypobranchial plate by a wide strip of cartilage, Cb I bears a wide and flattened *p. anterior branchialis* on its anterior margin. Ceratobranchials II, III and IV are fused to the hypobranchial plate, the former two via a narrow bar of cartilage and Cb IV via a wider cartilaginous connection. Only ceratobranchial I and II bear poorly developed *spiculae*, near their point of attachment to the hypobranchial plate.

DISCUSSION

The tadpoles of *Eupsophus queulensis* are very similar in morphology and color patterns to those of *E. roseus*, *E. emihopugini*, *E. calcaratus* and *E. vertebralis* (FORMAS & PUGIN, 1978, FORMAS, 1989a-b, 1992; VERA CANDIOTI et al., 2005). They have elliptical body, approximately two times longer than higher, their tail length is almost twice the body length. Eyes are positioned dorsolaterally. The creamy white color and scant pigmentation are common traits of endotrophic midcolored tadpoles (THIBAudeau & ALTIG, 1999).

Eupsophus tadpoles differ in a few characteristics. The spiracular tubes of *Eupsophus emihopugini* and *E. vertebralis* are short, sinistral and ventrolaterally located (FORMAS, 1989a, 1992). *Eupsophus queulensis*, *E. roseus* (FORMAS & PUGIN, 1978) and *E. calcaratus* (FORMAS, 1989b) share a small and sinistral spiracular opening without spiracular tube. A single, sinistral, and ventral spiracular tube with a visible opening has been previously reported for *E. calcaratus* (VERA CANDIOTI et al., 2005). Tadpoles of *E. calcaratus* (IZUA 2896-2897, 2957) examined herein do not have spiracular tube. The lack of a spiracular tube is a modification for tadpoles with midcolored life history (THIBAudeau & ALTIG, 1999).

The oral disc of *E. queulensis* and all described *Eupsophus* larvae does not differ from that of typical exotrophic larvae. It is ventral and has a single row of marginal papillae with a

distinct rostral gap. The reported labial tooth row formulae range from 2/2 in *E. roseus* (FORMAS & PUGIN, 1978), *E. emihopugni* (FORMAS, 1989a) and *E. calcaratus* (FORMAS, 1989b) to 2(2)/2 in *E. queulensis* (this work) and 2(2)/2(1) in *E. vertebralis* (FORMAS, 1992). The presence of a vestigial third posterior row in one individual and the following intraspecific variation was reported for larvae of *E. calcaratus*, 2(2)/2, 2(2)/2(1) (VERA CANDIOTI et al., 2005).

Only the chondrocranium of *Eupsophus calcaratus* had been described so far (VERA CANDIOTI et al., 2005). Herein we compare the main differences between *E. queulensis* (stage 34) and *E. calcaratus* (stage 31); characteristics for *E. calcaratus* are given in parenthesis. *Eupsophus queulensis* has body and wings of the suprarostals largely fused (joined by a cartilaginous dorsal bridge), the trabecular horns are of uniform width throughout their length (anteriorly narrow); the trochlear foramen is absent (present); the *p. ascendens* of the palatoquadrate has an intermediate attachment to the braincase (low attachment); the infrarostrals are connected medially through connective tissue (infrarostrals fused into a single element), *commissurae terminales* are absent between Cb I and Cb II (present), and *spiculae* absent in Cb III and Cb IV (present in Cb III).

Based on adult morphology, karyotypes, call characteristics, and a recent molecular phylogeny, the ten species currently included in the genus *Eupsophus* have been separated into two species groups, the *roseus* and the *vertebralis* groups (FORMAS 1980, 1991, 1993; FORMAS et al., 1983, 1992, FERNANDEZ DE LA RIGUERA, 1987, PENNA & VILLOSO, 1990; ORTIZ & IBARRA-VIDAL, 1992; PENNA & SOLÍS, 1995; NÚÑEZ et al., 1999; NÚÑEZ, 2003). The available chondrocranial data are restricted to two species of the *roseus* group. Given the lack of baseline tadpole data, it is not yet possible to determine if larval characteristics (external and internal) can provide additional support to differentiate the two groups.

RESÚMEN

El género *Eupsophus* se caracteriza por poseer larvas endotróficas que habitan cavidades con agua. Durante la metamorfosis las larvas se sustentan de sus grandes reservas vitelinas. La morfología externa ha sido descrita para cuatro de las diez especies conocidas en el género; características de la anatomía condrocraNeal fueron reportadas sólo para una especie. En este trabajo se describen la morfología larval externa, el disco oral larval y la anatomía condrocraNeal de *Eupsophus queulensis*. Las características de la larva de esta especie se comparan con las de otras larvas en el género *Eupsophus*.

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